The effect of dietary onion and garlic on hepatic lipid concentrations and activity of antioxidative enzymes in chicks

D. Sklan, Y.N. Berner, and H.D. Rabinowitch

Faculty of Agriculture, Hebrew University, Rehovot 76-100, Israel

Chickens were fed 2% lyophilized onion or garlic with different concentrations of disulfides in diets containing 18% soybean oil for 14 days. Hepatic S levels increased almost two-fold in chicks fed garlic. Hepatic cholesterol concentrations were reduced by garlic but not by onions. Total superoxide dismutase (SOD) activity was decreased by garlic and this was due to reduced activity of the Cu-Zn, SOD. However, in contrast, both onion and garlic increased Mn-SOD activity and hepatic glutathione concentrations. Glutathione peroxidase activity was decreased by garlic. These findings indicate that dietary garlic is more effective as a hypocholesterolemic agent than onion and has additional effects in reducing the activity of antioxidative enzymes. These effects are similar with disulfide intake from onion or garlic.

Keywords: onion; garlic; disulfides; cholesterol; SOD; glutathione

Introduction

Onions and garlic are regarded in many areas as folk medications. Recently, research has accumulated suggesting that these two alliaceous plants have beneficial effects when included in the diet. This has been reviewed recently in detail by Fenwick and Hanley[1] and Augusti.[2]

One area where alliums have been shown to have some effect is blood and tissue lipid levels. Cardiovascular disease is a major cause of mortality in western society, and high circulating cholesterol levels are considered as a major risk factor in atherogenesis. Epidemiological studies have also demonstrated correlations between circulating lipid levels and morbidity in several kinds of cancer. Garlic and onion have hypolipidemic effects in humans, rats, and rabbits in both serum and tissues.[3,4] This effect has been attributed to the aliphatic disulfides, in particular allicin, in both onions *(Allium cepa L.)* and garlic *(A. sativum L.)*. These materials contain alliins, cysteine sulfoxide compounds, that are converted by alliinase when the vegetable cells are crushed to allicin (a disulfide oxide) while releasing pyruvic acid.[5] These compounds have been shown to inactivate −SH groups and cause oxidation of NADPH,[6,7] and are present at higher levels in garlic than onions,[8] but their influence on oxidative reactions in vivo has not been examined.

This study was designed to examine the effects of garlic and onions fed at intake/body weight levels similar to that previously reported[9] on hepatic lipid metabolism and on some antioxidative enzymatic activities in chicks.

Materials and methods

The plant materials used were onion *cv.* RAM 710 (Hazera Seed Co., Haifa, Israel) and garlic *cv.* Shani. Plants were grown at the experimental farm, Faculty of Agriculture, Rehovot, Israel. Standard agricultural procedures were used throughout. Plants were harvested on maturation and cured for 7 days under ambient conditions before lyophilization. Relevant bulb composition is given in *Table 1*.

Six-day-old Leghorn × New Hampshire male chicks were fed the following basic diets that were formulated according

This study was supported in part by a Wolf Foundation grant of the Israel Academy of Science.
Address reprint requests to Dr. D. Sklan at the Faculty of Agriculture, Hebrew University, P.O. Box 12, Rehovot 76-100, Israel.
Received February 18, 1991; accepted October 17, 1991.

Table 1 Composition of onion and garlic bulbs

	Onion	Garlic
Dry matter (%)	10.4 ± 0.1	32.5 ± 0.5
Total soluble solids (%)	8.4 ± 0.1	25.1 ± 0.4
Total pyruvate (μmol/g)	35.1 ± 2.4	240.0 ± 12.5

Table 2 Feed concentrations of S and metals

	Control	Onion	Garlic
S (mg/g)	6.01	6.16	6.26
Cu (μg/g)	17.1	16.9	16.9
Zn (μg/g)	91.0	89.8	90.0
Mn (μg/g)	119.5	117.3	117.4

to NRC.[10] The diets contained (%): sorghum 30.5, soybean meal (45% protein) 40.7, soybean oil (no additives) 18.0, methionine 0.3, calcium carbonate, 3.3, dicalcium phosphate 4.2, and vitamin and mineral mix 1.0;[11] an additional 2% lyophilized onion powder, garlic powder, or sorghum was added. These diets contained 3.39 Mcals/kg ME (calculated), 21.3% protein, 0.67% methionine, and 1.01% total sulphur amino acids. Tap water was available ad libitum. Birds were maintained in a battery brooder. Dietary metals and sulphur concentrations are given in *Table 2*.

After 14 days on the experimental diets, blood was taken into heparinized tubes and birds killed by an intracardiac overdose of sodium pentobarbital. Liver was removed and a portion homogenized for enzymatic determinations or stored at −20° C until analysis.

Superoxide dismutase (SOD, EC 1.15.1.1) activity in liver homogenates was determined as described by Beauchamp and Fridovich,[12] with 50 μmol/L xanthine and sufficient xanthine oxidase to cause an A_{550} of 0.025/min at pH 7.8 and 25° C in a total reaction volume of 3 mL. SOD activity was also determined in an aliquot of homogenate treated with CN^- to inactivate the Cu-Zn, SOD. The unit of SOD activity is described as the amount of enzyme that inhibited the reaction rate by 50% under our experimental conditions. Glutathione peroxidase (Glu-px, EC 1.11.1.9) activity towards hydrogen peroxide was determined as described by Awasthi et al.[13] using 4 mmol/L glutathione. Activity was calculated from the change in concentration of NADPH. One unit of enzyme brings about the oxidation of 1 μmol of glutathione per min at 37° C.

Alliins were estimated by the pyruvate development procedure as described by Schwimmer and Weston[14] and soluble solids were determined with a thermo-regulated refractometer.[15,16]

Lipids were extracted with chloroform-methanol,[17] and cholesterol[18] and triglycerides[19] were determined colorimetrically. Amino acids were determined after hydrolysis at 110° C for 20 hrs with 6 N HCl with or without pretreatment with performic acid.[20]

Metals and sulphur were determined by inductive coupled argon plasma spectrometry (ICP). Samples were oven dried at 100° C for 3 hr followed by dry heating to 550° C for 3 hr until fine white ash was obtained. Super-Q distilled water was added containing 0.8% HCl and 2.5% nitric acid, the solution was heated until ash disappeared, and diluted to 25 mL. Elements were determined using an ICP (Spectroflame, Klever, Germany.[21]

Analysis of variance was performed by standard methods and the differences between treatments determined by examining all values of the hypothesis H_0: LSM (i) = LSM(j).[22]

Results

No difference in body weight gain between treatments were observed and liver weights did not differ *(Table 3)*. Feed conversion (feed/gain) was not different between the treatments and varied between 2.22 and 2.23 kg/kg.

Concentrations of Cu, Zn, and Mn in the liver are shown in *Figure 1* together with concentrations of S. Diets did not effect hepatic concentrations of metals. S levels in liver were increased almost two-fold in chicks fed garlic, whereas feeding onions had no effect.

Hepatic cholesterol concentrations *(Figure 2)* were decreased by garlic but not by onions. On the other hand, triglyceride concentrations were not influenced by garlic but were slightly increased by onions. Phospholipid levels (not shown) did not change.

Table 3 Weight gain and liver weights at the end of the experimental period

	Control	Onion	Garlic
Weight gain (g)	99.8 ± 7.2	107.3 ± 6.8	98.5 ± 7.1
Liver weight (g)	6.7 ± 0.4	6.9 ± 0.5	6.6 ± 0.5

Results are means ± SD from 10 birds.

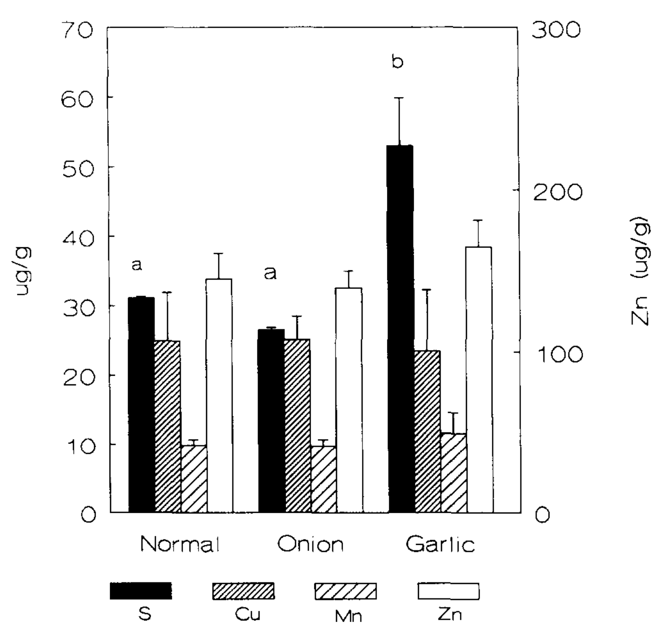

Figure 1 Hepatic concentrations of copper, manganese, zinc, and sulphur (μg/g) wet weight in chicks fed control, onion-containing, or garlic-containing diets. Results are from 10 chicks. Bars represent SD. Bars not marked by the same letter differ significantly (*P* < 0.01).

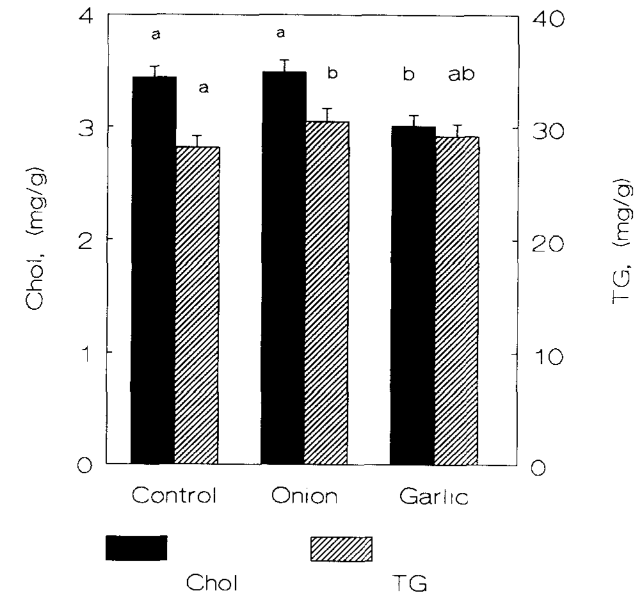

Figure 2 Hepatic concentrations of cholesterol and triglycerides (mg/g) in chicks fed control, onion-containing, or garlic-containing diets. Results are from 10 chicks and bars represent SD. Bars not marked by the same letter differ significantly ($P < 0.05$).

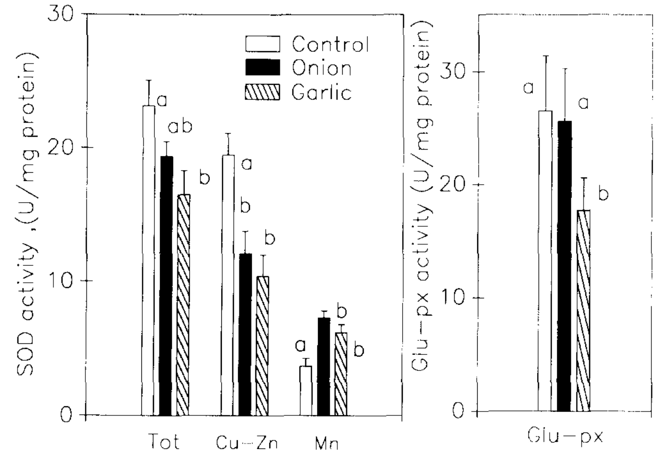

Figure 3 Hepatic activity of total, Cu-Zn, and Mn-SOD (left panel) and of Glu-px (right panel) in chicks fed control or onion or garlic containing diets. Results are from 10 chicks. Bars represent SD. Bars not marked by the same letter differ significantly ($P < 0.05$).

Activities of total, Cu-Zn, and Mn SOD are shown in *Figure 3.* Total SOD activity tended to decrease when onion was fed and was reduced by one-third when garlic was included in the diet. Examination of the separate SOD activities showed that this decrease was due to extensively decreased activity of the Cu-Zn, SOD; whereas, Mn-SOD exhibited increased activity by up to two-fold with both onion and garlic. Glu-px activity was also considerably decreased when garlic was fed but onion had no effect on this enzyme *(Figure 3).*

Hepatic glutathione concentrations increased and both sulphur amino acids tended to increase when either garlic or onion were included in the ration *(Table 4).*

Discussion

This study demonstrated hypocholesterolemic effects and reduced antioxidative enzymes activity in chicks fed garlic, but not in those fed onions. These effects correspond to the relative levels of disulfide compounds in the alliaceous supplements.

Hepatic cholesterol synthesis makes the greatest contribution to cholesterol turnover in the chicken.[23] In the present study no cholesterol was present in the diet, thus the hepatic cholesterol concentrations represent the sum of endogenous synthesis, recycling, and transport from the liver. Garlic, but not onion, was found to depress hepatic cholesterol levels in chicks, although onions have been reported to have this effect in humans and rabbits.[4,24,25] Hepatic TG concentrations did not appear to be affected by garlic, suggesting that overall transport of lipids was probably not decreased. These findings may indicate that feeding garlic could down-regulate liver cholesterol synthesis. Similarly, Jain[26] reported that garlic, but not onion juice, could ameliorate cholesterol-fed atherosclerosis in rabbits. Feeding garlic to rats has been shown to decrease hepatic cholesterol synthesis from acetate.[27] Mahanta et al.[28] reported that feeding 50 g raw garlic daily to humans had hypocholesterolemic effects in plasma; this quantity is similar on a body-weight basis to that used in the present study. The daily turnover of about 1.5 g cholesterol in humans is derived by approximately one-third exogenous absorption and two-thirds endogenous production.[29] Thus, extrapolating from the results reported here, the decrease in circulating cholesterol in humans by feeding them garlic may be the result of decreased liver cholesterol synthesis.

Both onions and garlic contain sulphur as polysulfides, whereas garlic has markedly higher concentrations. Chicks fed onion showed no change in overall liver S content, whereas garlic-fed chicks had almost twice the liver S content. This was in contrast to the relatively small differences in total dietary content, and may be due to enhanced absorption of alliaceous S. Dietary inorganic sulphate is converted in part to cysteine in chicks[30] and this may explain the tendency to the increased S amino acids and glutathione observed here.

One of the described effects of onions and garlic is

Table 4 Hepatic glutathione and sulfur amino acid concentrations

	Control	Onion	Garlic
Glutathione (μmol/μg prot)	8.60 ± 1.81[a]	12.75 ± 2.33[b]	11.87 ± 1.86[b]
Cysteine %	4.59 ± 0.61	5.66 ± 0.60	5.23 ± 0.53
Methionine %	1.79 ± 0.18	1.98 ± 0.19	1.92 ± 0.22

Results are means ± SD from 10 birds. Values in rows not followed by the same letter differ significantly ($P < 0.05$).

due to the reaction of disulfides with SH groups and NADPH, which are required for cholesterol and fatty acid synthesis, causing inactivation by thiol disulfide reactions and oxidation.[3] In this study we observed that garlic enhanced hepatic glutathione concentration, whereas Glu-px activity decreased concomitantly. This indicates less overall oxidative activity, as does the decreased total SOD activity in these animals. However, Mn-SOD increased, and as this enzyme is located in the mitochondria it may indicate enhanced oxidative activity within the mitochondria together with reduced cytosolic oxidative activity. These differences in SOD activities did not appear to be due to any changes in the concentrations of elements in the metal core of SOD, as indicated by total Cu, Zn, or Mn concentrations in the liver. Under these conditions, feeding onion did not produce any significant changes from the control chicks.

The overall effect of the dietary garlic would seem to be a reduction in activity of antioxidative enzymes in cytosol. This could be due to a sparing of these activities as the enhanced levels of glutathione and sulphur amino acids can react directly with oxidizing compounds.[30]

The effects of garlic demonstrated here were greater than those found with onions both on cholesterol levels and on antioxidative enzymes activity, and are in parallel with S-alliaceous content in the diet. These findings correlate with previous findings in other species[23] and suggest beneficial influences of high dietary garlic levels.

Acknowledgments

We would like to thank O. Greenberg and O. Kedar for their skilled technical assistance.

References

1 Fenwick, G.R. and Hanley, A.B. (1985). Genus allium -Part 3. *CRC Critical Reviews Food Science and Nutrition, Vol 23*, p. 1-73, CRC Press, Boca Raton, FL, USA

2 Augusti, K.T. (1990). Therapeutic and medicinal values of onions and garlic. In *Onions and Allied Crops*, Vol 3, (J.L. Brewster and H.D. Rabinowitch, eds.), p. 93-108. CRC Press, Boca Raton, FL, USA

3 Adamu, I., Joseph, P.K., and Augusti, K.T. (1982). Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two month period. *Experimentia* **38**, 899–902

4 Bordia, A., Arora, S.K., Kothari, L.K., Jain, K.C., Rathore, B.S., Dube, M.K., and Bhu, N. (1975). The protective action of essential oils of onion and garlic in cholesterol fed rabbits. *Atherosclerosis* **22**, 103–109

5 Smittle, D.A., Hayes, M.J., and Dickens, W.L. (1979). Quality evaluation of onion. *Univ. Georgia Res. Report* **336**, 1–10

6 Katzen, H.M. and Tietz, F. (1966). Studies on the specificity and mechanism of the action of glutathione insulin transhydrogenase. *J. Biol. Chem.* **241**, 3561–3565

7 Virtanen, A.I. and Matikkala, E.J. (1959). The isolation of S-methyl cysteine sulfoxide and s-n-propyl cysteine sulfoxide from onion *(Allium cepa)* and the antibiotic activity of crushed onion. *Acta Chem. Scand.* **13**, 1898–1905

8 Coley-Smith, J.R. (1986). A comparison of flavour and odour compounds of onion, leek, garlic and *Allium fistulosum* in relation to germination of scelerotia of *Scelerotium cepivorum. Plant Pathol.* **35**, 370–376

9 Sainani, G.S., Desai, D.B., Gorhe, N.H., Natu, S.N., Pise, D.V., and Sainini, P.G. (1979). Effect of dietary garlic and onion on serum lipid profile in Jain Community. *Ind. J. Med. Res.* **79**, 776–785

10 Nutrient Requirements of Poultry. (1984). National Academy Press, Washington, DC, USA

11 Sklan, D., Halevy, O., and Donoghue, S. (1987). The effect of different dietary levels of vitamin A on metabolism of copper iron and zinc in the chick. *Int. J. Vit. Nutr. Res.* **57**, 11–18

12 Beauchamp, C. and Fridovitch, I. (1971). Superoxide dismutase: improved assay and an assay applicable to polyacrylamide gels. *Anal. Biochem.* **44**, 276–287

13 Awasthi, Y.C., Beutler, E., and Srivastava, S.K. (1975). Purification and properties of human erythrocyte glutathione peroxidase. *J. Biol. Chem.* **250**, 5144–5149

14 Schwimmer, S. and Guadagni, D.G. (1962). Relation between olfactory threshold concentration and pyruvic acid content of onion juice. *J. Food Sci.* **27**, 94–97

15 Mann, L.K. and Hoyle, B.J. (1945). Use of the refractometer for selecting onion bulbs high in dry matter for breeding. *Proc. Am. Soc. Hort. Sci.*, **46**, 285–292

16 Nieuwhof, I.M., De Bruyn, J.W., and Garretsen, F. (1973). Methods to determine solidity and dry matter content of onions *(Alium Cepa L.). Euphytica* **22**, 39–47

17 Folch, J., Lees, M., and Sloan-Stanley, G.H.S. (1956). A simple method for the isolation and purification of lipides from animal tissues. *J. Biol. Chem.* **226**, 497–501

18 Searcy, R.L. and Bergqist, L.M. (1960). A new color reaction for the quantitation of serum cholesterol. *Clin. Chimia Acta.* **5**, 192–196

19 Sastry, P.S. and Kates, M. (1964). Determination of glycerol in glycosyl diglycerides and glycerides. *Biochemistry* **6**, 1271–1274

20 Halevy, O. and Sklan, D. (1984). A low molecular weight zinc-copper lipolytic protein from chick liver. *Life Sci.* **34**, 1945–1951

21 Ward, A.F., Marciello, L.F., Carrara, L., and Luciano, V.J. (1980). Simultaneous determination of major, minor and trace elements in agricultural and biological samples by inductively coupled argon plasma spectroscopy. *Spect. Letters* **13**, 803–831

22 Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods. 6th Ed.* Iowa State University Press, Ames, IA, USA

23 Sklan, D., Dahan, M., Budowski, P., and Hurwitz, S. (1977). Differential absorption of endogenous and exogenous cholesterol in the chick as affected by dietary oil and phytosterols. *J. Nutr.* **107**, 1996–2001

24 Augusti, K.T. and Mathew, P.T. (1973). Effect of long term feeding on the aqueous extracts of onions and garlic on normal rats. *Ind. J. Exp. Biol.* **11**, 239–246

25 Sharma, K.K., Chowdhury, N.K., and Sharma, A.L. (1975). Long term effect of onion on experimentally induced hypercholesteremia and consequently decreased fibrinolytic activity in rabbits. *Ind. J. Med. Res.* **63**, 1629–1638

26 Jain, R.C. (1976). Onion and garlic in experimental cholesterol induced atherosclerosis. *Ind. J. Med. Res.* **64**, 1509–1515

27 Chang, M.L.W. and Johnson, M.A. (1980). Effect of garlic on carbohydrate mechanism and lipid synthesis. *J. Nutr.* **110**, 931–936

28 Mahanta, R.K., Goswami, R.K., Kumar, D., and Goswami, P. (1980). Effect of *Allium sativum* (garlic) on blood lipids. *Ind. Med. Gazette* (Apr.), 157

29 Miettinen, T.A. and Kesaniemi, Y.A. (1989). Cholesterol absorption: regulation of cholesterol synthesis and elimination and within population variations of serum cholesterol levels. *Am. J. Clin. Nutr.* **49**, 629–635

30 Bornstein, S. and Plavnik, Y. (1977). The sparing action of inorganic sulphate on sulphur amino acids in practical broiler diets: preliminary trials with young chicks. *Br. Poult. Sci.* **18**, 19–31